

BB-1174

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)

09/744100

INTERNATIONAL APPLICATION NO.

PCT/US99/15931

INTERNATIONAL FILING DATE

14 JULY 1999 (14.07.99)

PRIORITY DATE CLAIMED

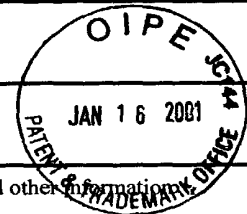
17 JULY 1998 (17.07.98)

TITLE OF INVENTION

ORNITHINE BIOSYNTHESIS ENZYMES

APPLICANT(S) FOR DO/EO/US

CAHOON, Rebecca E., et al.



Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to bring national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b)) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19<sup>th</sup> month from the earliest claimed priority date.
5. ☒ A copy of the International Application was filed (35 U.S.C. 371 (c) (2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371 (c) (2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c) (3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

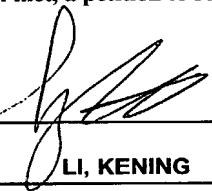
**Items 13 to 18 below concern document(s) or information included :**

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.  
A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☒ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail.
19. ☐ Other items or information:

17. General Power of Attorney

18. Express Mailing Label No.: EL031052277US

*mailed  
1/16/01*

APPLICATION NO. (IF KNOWN, SEE 37 CFR)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/744100		PCT/US99/15931		BB-1174	
20. The following fees are submitted				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO				\$860.00	
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482)				\$690.00	
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))				\$760.00	
<input type="checkbox"/> Neither international preliminary examination fee paid to USPTO (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO				\$1000.00	
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) And all claims satisfied provisions of PCT Article 33(2)-(4)				\$ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	7 - 20 =	0 x	\$18.00	\$0.00	
Independent Claims	3 - 3 =	0 x	\$80.00	\$0.00	
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>				\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$0.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input type="checkbox"/>				\$0.00	
SUBTOTAL =				\$0.00	
Processing Fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
TOTAL NATIONAL FEE =				\$860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input checked="" type="checkbox"/>				\$40.00	
TOTAL FEES ENCLOSED =				\$900.00	
				Amount to be : refunded	\$
				Charged	\$
<input type="checkbox"/> A check in the amount of _____ to cover the above fees enclosed.					
<input checked="" type="checkbox"/> Please charge my Deposit Account No. 04-1928 in the amount of \$900.00 to cover the above fees.					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 04-1928 a duplicate copy of this sheet is enclosed.					
NOTE : Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (CFR 1.37(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
LI, KENING E. I. DU PONT DE NEMOURS AND COMPANY Legal Patent Records Center 1007 Market Street Wilmington, Delaware 19898 United States of America			SIGNATURE  NAME LI, KENING 44,872 REGISTRATION NUMBER 1/15/2001 DATE		

TITLE

ORNITHINE BIOSYNTHESIS ENZYMES

This application claims the benefit of U.S. Provisional Application No. 60/093,209, filed July 17, 1998.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding N-acetyl glutamate kinase in plants and seeds.

BACKGROUND OF THE INVENTION

Ornithine is converted into arginine in the urea cycle. Intermediaries in the ornithine biosynthesis pathway are important in other steps of this cycle. Amino acid N-acetyl transferase (EC 2.3.1.1) catalyzes the first reaction in a pathway that leads to the synthesis of ornithine from L-glutamate giving N-acetylglutamate as its intermediary product. Carbamoyl phosphate synthase I, the mitochondrial enzyme that catalyzes the first committed step of the urea cycle, is allosterically activated by N-acetyl glutamate. The rate of urea production by the liver is, in fact, correlated with the N-acetylglutamate concentration. Increased urea synthesis is required when amino acid breakdown rates increase, generating excess nitrogen that must be extracted. Increase in these breakdown rates are signaled by an increase in glutamate concentration through transamination reaction. This situation, in turn, causes an increase in N-acetylglutamate synthesis, stimulating carbamoyl phosphate synthetase and the entire urea cycle.

N-acetyl glutamate kinase (EC 2.7.2.8) catalyzes the conversion of N-acetyl-L-glutamate and ATP into N-acetyl-L-glutamate-5-phosphate and ADP. N-acetylglutamate kinase is encoded by the *argB* locus in bacteria and *Synechocystis*. This enzyme has been characterized at the molecular level in the red alga *Porphyra umbilicalis*, a member of the protista kingdom (Reith, M., Munholland, J. (1993) *Curr. Genet.* 23:59-65), but it has yet to be described in higher plants.

SUMMARY OF THE INVENTION

The instant invention relates to isolated nucleic acid fragments encoding N-acetyl glutamate kinase. Specifically, this invention concerns an isolated nucleic acid fragment encoding an N-acetyl glutamate kinase and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding an N-acetyl glutamate kinase. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding N-acetyl glutamate kinase.

An additional embodiment of the instant invention pertains to a polypeptide encoding all or a substantial portion of an N-acetyl glutamate kinase.

In another embodiment, the instant invention relates to a chimeric gene encoding an N-acetyl glutamate kinase, or to a chimeric gene that comprises a nucleic acid fragment that

is complementary to a nucleic acid fragment encoding an N-acetyl glutamate kinase, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

5 In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding an N-acetyl glutamate kinase, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived  
10 from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the level of expression of an N-acetyl glutamate kinase in a transformed host cell comprising:  
15 a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an N-acetyl glutamate kinase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of N-acetyl glutamate kinase in the transformed host cell.

20 An addition embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding an N-acetyl glutamate kinase.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an N-acetyl glutamate kinase, the method  
25 comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an N-acetyl glutamate kinase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of N-acetyl glutamate kinase in the transformed host cell; (c) optionally  
30 purifying the N-acetyl glutamate kinase expressed by the transformed host cell; (d) treating the N-acetyl glutamate kinase with a compound to be tested; and (e) comparing the activity of the N-acetyl glutamate kinase that has been treated with a test compound to the activity of an untreated N-acetyl glutamate kinase, thereby selecting compounds with potential for inhibitory activity.

35 BRIEF DESCRIPTION OF THE  
DRAWING AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawing and Sequence Listing which form a part of this application.

Figure 1 depicts the amino acid sequence alignment between the N-Acetylglutamate Kinase from *Synechocystis* sp. (NCBI General Identifier No. 1652434: SEQ ID NO:11), the instant corn contig assembled of clones cr1n.pk0113.g3 and p0111.cipmf81r (SEQ ID NO:2), the instant rice clone rls72.pk0023.c6 (SEQ ID NO:4), the instant soybean clone sdp2c.pk010.h6 (SEQ ID NO:6) and the instant wheat contig assembled of clones wlk1.pk0019.d6 and w11.pk0009.a7. The top row indicates with asterisks (\*) the amino acids conserved among all sequences and with plus signs (+) the amino acids conserved only among the plant sequences. Dashes are used by the program to maximize the alignment of the sequences. The bottom row contains the consensus sequence (SEQ ID NO:12) comprising all the amino acids which are conserved among all plant sequences.

Table 1 lists the polypeptides that are described herein, the plant from which the clones originated, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1  
N-Acetylglutamate Kinase

Plant	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Corn	Contig of: p0111.cipmf81r cr1n.pk0113.g3	1	2
Rice	rls72.pk0023.c6	3	4
Soybean	sdp2c.pk010.h6	5	6
Wheat	Contig of: wlk1.pk0019.d6' w11.pk0009.a7	7	8
Wheat	wr1.pk0080.h2: fis	9	10

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a “nucleic acid fragment” is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid  
5 fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

As used herein, “contig” refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid  
10 fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, “substantially similar” refers to nucleic acid fragments wherein  
15 changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. “Substantially similar” also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-  
20 suppression technology. “Substantially similar” also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention  
25 encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100%  
30 sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more  
35 hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which

result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

5        Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) *Nucleic Acid Hybridisation*, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar  
10       fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min. then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min. and then repeated twice with  
15       0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2x SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

20       Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Preferred are those nucleic acid fragments whose nucleotide sequences encode amino acid sequences that are 80% identical to the amino acid sequences  
25       reported herein. More preferred nucleic acid fragments encode amino acid sequences that are 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite  
30       (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

35       A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-

based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool: Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively

5 identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or  
10 more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that  
15 comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

20 "Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited  
25 by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building  
30 blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments  
35 may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan



appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences

have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide.

WO 00/04168

PCT/US99/15931

“Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. “Co-suppression” refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

“Altered levels” refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

“Mature” protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. “Precursor” protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A “chloroplast transit peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. “Chloroplast transit sequence” refers to a nucleotide sequence that encodes a chloroplast transit peptide. A “signal peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

“Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Maniatis”).

Nucleic acid fragments encoding at least a portion of several N-acetylglutamate kinases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST

algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other N-acetylglutamate kinases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673; Loh et al. (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing

portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest  
5 (Lerner (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of arginine in those cells. Extra arginine  
10 resulting from an increase in ornithine biosynthesis may have nutraceutical utility. Also, N-acetylglutamate kinase is a potential herbicide target and may be the site of TZO-type chemistry. Overexpression of the gene coding for this enzyme, isolation of the enzyme and introduction into a screen based on its activity, may provide a means of identifying chemistry with herbicidal or fungicidal activity.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter  
15 sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the  
25 plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired  
30 expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptide to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that  
35 the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptide with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant*

*Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

5 It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptide in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the  
10 instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression  
15 have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U. S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of  
20 gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in  
25 which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the  
30 skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen  
35 by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of

samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptide (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptide of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptide are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptide. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded N-acetylglutamate kinase. An example of a vector for high level expression of the instant polypeptide in a bacterial host is provided (Example 6).

Additionally, the instant polypeptide can be used as a target to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptide described herein catalyzes a step in ornithine biosynthesis. Accordingly, inhibition of the activity of the enzyme described herein could lead to inhibition plant growth. Thus, the instant polypeptide could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the

methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

5 Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

10 In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

15 A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080),  
20 nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those  
25 skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

30 Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al. (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may  
35 be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the



mutation tag element in or near the plant gene encoding the instant polypeptide.

Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptide can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptide disclosed herein.

### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

#### EXAMPLE 1

##### Composition of cDNA Libraries: Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean and Wheat

Library	Tissue	Clone
cr1n	Corn Root From 7 Day Old Seedlings*	cr1n.pk0113.g3
p0111	Corn V6 Stage Leaf Tissue Minus Midrib + UV, screened 1 Pool of PR + UV 3h; PR +UV 24 h; PR+UV 48h; and PR+UV 7 days	p0111.cipmf81r
rls72	Rice Leaf 15 Days After Germination, 72 Hours After Infection of Strain <i>Magaporthe grisea</i> 4360-R-67 (AVR2-YAMO); Susceptible	rls72.pk0023.c6
sdp2c	Soybean Developing Pods (6-7 mm)	sdp2c.pk010.h6
w11	Wheat Leaf From 7 Day Old Seedling	w11.pk0009.a7
wk1	Wheat Seedlings 1 Hour After Treatment With Herbicide**	wk1.pk0019.d6
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk0080.h2

\*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

\*\*Application of 6-iodo-2-propoxy-3-propyl-4(3H)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### EXAMPLE 2

##### Identification of cDNA Clones

cDNA clones encoding N-acetylglutamate kinase were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3Characterization of cDNA Clones Encoding N-Acetylglutamate Kinase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to N-acetylglutamate kinase from *Synechocystis sp.* (NCBI General Identifier No. 1652434). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs or assembled of an FIS and an EST ("Contig"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous  
to N-Acetylglutamate Kinase

Clone	Status	BLAST pLog Score 1652434
Contig of: p0111.cipmf81r cr1n.pk0113.g3	Contig	102.0
rls72.pk0023.c6	FIS	94.1
sdp2c.pk010.h6	FIS	95.0
Contig of: wlk1.pk0019.d6 wl1.pk0009.a7	Contig	86.0
wr1.pk0080.h2	FIS	29.0

The nucleotide sequence of wheat clone wlk1.pk0019.d6 was missing 51 nucleotides. To complete the entire sequence of a wheat N-acetylglutamate kinase 51 nucleotides were added as unknowns (n) and converted into 17 unknown amino acids (X, amino acids 144 to 160). Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6 and 8 and the *Synechocystis sp.* sequence (SEQ ID NO:11). The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8 and 10 and the *Synechocystis sp.* sequence (SEQ ID NO:11). The amino acid sequence set forth in SEQ ID NO:10 is 89% identical over the 98 amino acid region or overlap with the amino acid sequence set forth in SEQ ID NO:8.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences  
of cDNA Clones Encoding Polypeptides Homologous  
to N-Acetylglutamate Kinase

SEQ ID NO.	Percent Identity to 1652434
2	60.6
4	56.6
6	57.6
8	52.5
10	59.4

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "consensus sequence" was assembled using all the amino acids conserved among the plant (corn, rice, soybean and wheat) sequences (SEQ ID NO:12). The amino acids vary among the different plants creating positions where more than one amino acid may be present. Table 5 presents a list of the positions where these amino acids vary and the possible amino acids present at these positions.

TABLE 5

Amino Acid Variability Among Plant N-Acetylglutamate Kinase

Amino Acid Position in Consensus Sequence	Possible Amino Acids
2	Leu, Met
3	Leu, Ala
4, 176	Thr, Ala, Gly
5	Pro, none
6	Trp, His, none
7	Leu, none
8, 9, 46	Ser, Ala, none
10	Ser, Lys, Pro
11	Lys, Ser, Thr, Ala

Amino Acid Position in Consensus Sequence	Possible Amino Acids
12	Leu, Phe
13	Pro, Thr. none
14	Val, Leu. Asn
15	Pro, Leu
16. 182	Ser, Cys
17. 36	Pro, Thr. Ala
18	Pro, Arg. Ser
19	Pro, Val. Phe, Leu
20. 30	Ser, Lys. Thr, Arg
21	Gly, Phe. Asn
23. 50	Thr, Ala. Asn
24	Leu, Pro. Lys
25	Ser, Gly, Pro, Lys
26	Ser, Pro. Gln, Ala
27	Asn, Ala
28	His, Gln. Arg
29	Ala, Leu, Val
31	Pro, Thr
32	Leu, Ile, Ser
33	Ala, His
34, 49, 51, 52, 74, 194, 252, 265, 309	Ala, Ser
35	Pro, Ser. Phe
37	Cys, Ala. Ser. Pro
38	Arg, Pro. Thr. His
39	Arg, Gly. none
40. 43	Arg, Leu
41	Ser, Arg
42	Arg, Cys. His, Gly
44	Arg, Ala
45. 253	Ile, Leu. Val
47	Ala, Val. none
48	Thr, Val. none
53	Pro, Gln. Ser
54. 57	Ser, Ala. Pro
55	Pro, Ala, Leu

Amino Acid Position in Consensus Sequence	Possible Amino Acids
56	Ser, Leu, Ala
59	Ala, Glu, Gln
60, 227, 428	Ala, Thr
62	Thr, Ser, none
63, 191, 197, 269, 291	Glu, Ala
64	Ala, Ser, Gly
65	Leu, Gln
66	Ser, Tyr, Asn
80, 82	Arg, Lys
86, 137, 154, 179, 226, 281	Val, Ile
106	Arg, Asn
117, 215, 308	Arg, His
133	Leu, Gly, Gln
135, 209	Val, Lys
136	Gly, Asn
138	Glu, Pro
139	Gln, Val
142, 210, 272	Asp, Asn
150	Leu, Asp
151, 190	Thr, Asn
156	Glu, Ser
161, 201	Gly, Arg
166	Asn, Glu, Thr, Gln
172	Asn, Lys, Arg
173	Ile, Leu, Lys, Pro
174, 211, 237	Ala, Pro
177, 228, 234	Thr, Ser
180, 221	Gly, Ser
183	Gly, Trp, Arg
184	Lys, Met
185, 292	Asp, Glu
186, 230, 296	Gly, Ala
189, 217, 249, 259, 261	Ile, Leu
196	Asn, Lys, Asp
198, 284	Lys, Ala

Amino Acid Position in Consensus Sequence	Possible Amino Acids
199	Ala, Gly, Asp
202	Phe, Tyr
205	Glu, Gly
207	Ser, Trp, Ala, Thr
212, 220	Thr, Ala, Ser
216	Pro, Ser
219	Ala, Asp
245	Ala, Val
275	Gly, Asp
280	Val, Glu, Lys
288	Lys, Gln, Arg
290	Val, Met
294	Lys, Gln
304	Glu, Asn, Gly
405	His, Ile

Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode an almost entire wheat

5 N-acetylglutamate kinase, entire corn, rice and soybean N-acetylglutamate kinases, and a substantial portion of a wheat N-acetylglutamate kinase variant. These sequences represent the first plant sequences encoding N-acetylglutamate kinase.

#### EXAMPLE 4

### Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptide in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas,

VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight,  
5 essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene  
10 encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptide, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10  
15 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum  
20 of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236)  
25 which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added  
35 to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles



resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

#### EXAMPLE 5

##### Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptide in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described

above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptide. To induce somatic embryos, cotyledons,  
5 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained  
10 as described below.

Soybean embryogenic suspension cultures can maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

15 Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a  
20 chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptide  
25 and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the  
30 supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an  
35 empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the

retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 6

##### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptide can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptide

are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio- $\beta$ -galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50  $\mu$ L of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One  $\mu$ g of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

#### EXAMPLE 7

##### Evaluating Compounds for Their Ability to Inhibit the Activity of N-Acetylglutamate Kinase

The polypeptide described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptide may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His<sub>6</sub>"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptide, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptide are expressed as fusion proteins, the purification protocol may include

- the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptide may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)<sub>6</sub> peptide may be engineered into the
- 5 N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin.
- 10 These reagents include  $\beta$ -mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.
- 15 Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptide disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for N-acetylglutamate kinase are presented by Wolf, E. C. and Weiss, R. L. (1980)
- 20 *J. Biol. Chem.* 255:9189-9195.

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding an N-acetylglutamate kinase comprising a member selected from the group consisting of:
  - 5 (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 95% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 and encoding a functional enzyme;
  - 10 (b) an isolated nucleic acid fragment that is complementary to (a).
2. The isolated nucleic acid fragment of Claim 1 wherein nucleic acid fragment is a functional RNA.
3. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group
  - 15 consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7.
4. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.
5. A transformed host cell comprising the chimeric gene of Claim 4.
6. An isolated nucleic acid fragment encoding an N-acetylglutamate kinase
  - 20 having the sequence set forth in SEQ ID NO:12, where:
    - Xaa at position 2 is Leu or Met,
    - Xaa at position 3 is Leu or Ala,
    - Xaa at position 4 or 176 is Thr, Ala or Gly,
    - Xaa at position 5 is Pro or none,
    - 25 Xaa at position 6 is Trp, His or none,
    - Xaa at position 7 is Leu or none,
    - Xaa at position 8, 9, or 46 is Ser, Ala or none.
    - Xaa at position 10 is Ser, Lys or Pro,
    - Xaa at position 11 is Lys, Ser, Thr or Ala,
    - 30 Xaa at position 12 Leu or Phe,
    - Xaa at position 13 is Pro, Thr or none.
    - Xaa at position 14 is Val, Leu or Asn,
    - Xaa at position 15 is Pro or Leu,
    - Xaa at position 16 or 182 is Ser or Cys,
    - 35 Xaa at position 17 or 36 is Pro, Thr or Ala,
    - Xaa at position 18 is Pro, Arg or Ser,
    - Xaa at position 19 is Pro, Val, Phe or Leu,
    - Xaa at position 20 or 30 is Ser, Lys, Thr or Arg,

Xaa at position 21 is Gly, Phe or Asn.  
Xaa at position 23 or 50 is Thr, Ala or Asn,  
Xaa at position 24 is Leu, Pro or Lys,  
Xaa at position 25 is Ser, Gly, Pro or Lys,  
5 Xaa at position 26 is Ser, Pro, Gln or Ala,  
Xaa at position 27 is Asn or Ala,  
Xaa at position 28 is His, Gln or Arg.  
Xaa at position 29 is Ala, Leu or Val.  
Xaa at position 31 is Pro or Thr,  
10 Xaa at position 32 is Leu, Ile or Ser.  
Xaa at position 33 is Ala or His,  
Xaa at position 34, 49, 51, 52, 74, 194, 252, 256 or 309 is Ala or Ser,  
Xaa at position 35 is Pro, Ser or Phe,  
Xaa at position 37 is Cys, Ala, Ser or Pro,  
15 Xaa at position 38 is Arg, Pro, Thr or His,  
Xaa at position 39 is Arg, Gly or none,  
Xaa at position 40 or 43 is Arg or Leu,  
Xaa at position 41 is Ser or Arg,  
Xaa at position 42 is Arg, Cys, His or Gly,  
20 Xaa at position 44 is Arg or Ala,  
Xaa at position 45 or 253 is Ile, Leu or Val,  
Xaa at position 47 is Ala, Val or none,  
Xaa at position 48 is Thr, Val or none,  
Xaa at position 53 is Pro, Gln or Ser,  
25 Xaa at position 54 or 57 is Ser, Ala or Pro,  
Xaa at position 55 is Pro, Ala or Leu.  
Xaa at position 56 is Ser, Leu or Ala,  
Xaa at position 59 is Ala, Glu or Gln,  
Xaa at position 60, 227 or 428 is Ala or Thr,  
30 Xaa at position 62 is Thr, Ser or none,  
Xaa at position 63, 191, 197, 269 or 291 is Glu or Ala,  
Xaa at position 64 is Ala, Ser or Gly,  
Xaa at position 65 is Leu or Gln.  
Xaa at position 66 is Ser, Tyr or Asn,  
35 Xaa at position 80 or 82 is Arg or Lys,  
Xaa at position 86, 137, 154, 179, 226, 281 is Val or Ile,  
Xaa at position 106 is Arg or Asn,  
Xaa at position 117, 215 or 308 is Arg or His.

Xaa at position 133 is Leu, Gly or Gln.  
Xaa at position 135 or 209 is Val or Lys,  
Xaa at position 136 is Gly or Asn.  
Xaa at position 138 is Glu or Pro,  
5 Xaa at position 139 is Gln or Val,  
Xaa at position 142, 210 or 272 is Asp or Asn.  
Xaa at position 150 is Leu or Asp,  
Xaa at position 151 or 190 is Thr or Asn,  
Xaa at position 156 is Glu or Ser,  
10 Xaa at position 161 or 201 is Gly or Arg,  
Xaa at position 166 is Asn, Glu, Thr or Gln,  
Xaa at position 172 is Asn, Lys or Arg,  
Xaa at position 173 is Ile, Leu, Lys or Pro,  
Xaa at position 174, 211 or 237 is Ala or Pro.  
15 Xaa at position 177, 228 or 234 is Thr or Ser.  
Xaa at position 180 or 221 is Gly or Ser,  
Xaa at position 183 is Gly, Trp or Arg,  
Xaa at position 184 is Lys or Met,  
Xaa at position 185 or 292 is Asp or Glu,  
20 Xaa at position 186, 230 or 296 is Gly or Ala.  
Xaa at position 189, 217, 249, 259 or 261 is Ile or Leu,  
Xaa at position 196 is Asn, Lys or Asp,  
Xaa at position 198 or 284 is Lys or Ala,  
Xaa at position 199 is Ala, Gly or Asp,  
25 Xaa at position 202 is Phe or Tyr,  
Xaa at position 205 is Glu or Gly,  
Xaa at position 207 is Ser, Trp, Ala or Thr,  
Xaa at position 212 or 220 is Thr, Ala or Ser.  
Xaa at position 216 is Pro or Ser,  
30 Xaa at position 219 is Ala or Asp,  
Xaa at position 245 is Ala or Val,  
Xaa at position 275 is Gly or Asp,  
Xaa at position 280 is Val, Glu or Lys,  
Xaa at position 288 is Lys, Gln or Arg,  
35 Xaa at position 290 is Val or Met,  
Xaa at position 294 is Lys or Gln,  
Xaa at position 304 is Glu, Asn or Gly,  
and Xaa at position 405 is His or Ile.



7. A method for evaluating at least one compound for its ability to inhibit the activity of an N-acetylglutamate kinase, the method comprising the steps of:

- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an N-acetylglutamate kinase, operably linked to suitable regulatory sequences;
- (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the N-acetylglutamate kinase encoded by the operably linked nucleic acid fragment in the transformed host cell;
- (c) optionally purifying the N-acetylglutamate kinase expressed by the transformed host cell;
- (d) treating the N-acetylglutamate kinase with a compound to be tested; and
- (e) comparing the activity of the N-acetylglutamate kinase that has been treated with a test compound to the activity of an untreated N-acetylglutamate kinase,

thereby selecting compounds with potential for inhibitory activity.

(51) International Patent Classification <sup>7</sup> : C12N 15/54, 15/82, 9/12, 5/10, C12Q 1/68	A1	(11) International Publication Number: <b>WO 00/04168</b>
		(43) International Publication Date: 27 January 2000 (27.01.00)

**(81) Designated States:** AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(74) **Agent:** MAJARIAN, William, R.; E.I. du Pont de Nemours and Company, Legal Patent Record Center, 1007 Market Street, Wilmington, DE 19898 (US).

```

          *
SEQ ID NO:11 MSSTQDYIGE-----+
SEQ ID NO:02 MLLTKPYLNSLSLLVPVSPPPPSGPTLSSNHASPLAAPTCT-RSRLRISATSTAAPSPSSAA-
SEQ ID NO:04 MLLAKPILSSSSSF-LPSTRVSSPAPGNHAKPIAASPAP-RRCLRLAVTSAAPAASSAA-
SEQ ID NO:06 MMAG----AAKTLTNLCPSFPFPTKPQNQLTTSHAFPSTRLRHRAISAVANAQPPLAAA-
SEQ ID NO:08 MLLTKPH---PALTLPSASLPNPNLKAARVRPLASSAPHGRGRGRV---SASSSSSLAPAQ
SEQ ID NO:12 MXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXAX
              1                                     60

```

```

+      ++++++ ++++++ *++ ++++++*****+++++***** *+++++***** **
SEQ ID NO:11  ---EAATRVKILSEALPYIQHFAGRTVVVKYGGAAAMKDSNLKDKVIRDIVFMASVGIRPV
SEQ ID NO:02  AATASLRVDVLEALPFIQRFKGKTVVVKYGGAAAMKSPELQASVIRDLVLLSCVGLRPV
SEQ ID NO:04  AA-AALSRVDVLEALPFIQRFKGKTVVVKYGGAAAMKSPELQASVIRDLVLLSCVGLHPV
SEQ ID NO:06  TATEGQYRVDVLESPLPFIQKFRGKTIIVVKYGGAAAMKSPELQASVINDLVLLSCVGLRPV
SEQ ID NO:08  AASAALNRVDVLEALPFIQRFKGKTVVVKYGGAAAMKSPELQASVIRDLVLLSCVGLRPV
SEQ ID NO:12  AXXXXRVDVLEALPFIQFXFGKTXVVKYGGAAAMKSPELQASVIXDLVLLSCVGLXPV
61

```

```

*****+
SEQ ID NO:11 VVHGGGPEINTWLDKVGIEPQFKDGLRVTDAAATMDIVEMVLVGRVKNKELVNLIINQAGGKA
SEQ ID NO:02 LVHGGGPEINSWLLRVGVPEPQFRDGLRVTDALTMEVVMVLVGKVNKNLSLINTAGGTA
SEQ ID NO:04 LVHGGGPEINSWLLRVGVPEPQFRDGLRVTDALNMEVVMVLVKVNKELLSLIKLPGGS
SEQ ID NO:06 LVHGGGPEINSWLGRLNIPAVFRDGLRVTDADTMEIVSMVLVGKVNKTLVSLINKAGATA
SEQ ID NO:08 LVHGGGPEINSWLQRVGVXPQFRNGLRVTXXXXXXXXXXXXXXXXXQLLSLRPAGTTA
SEQ ID NO:12 LVHGGGPEINSWLXXXXXXFRXGLRVTDAXXMEVXXMVLVXKVNKXLSLIXXGXXA
121

```

This invention relates to an isolated nucleic acid fragment encoding an N-acetylglutamate kinase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the N-acetylglutamate kinase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the N-acetylglutamate kinase in a transformed cell host.

FIGURE 1

```

*      +
SEQ ID NO:11 MSSTQDYIGE-----+
SEQ ID NO:02 MLLTKPYLSNLLPVSPPPSGPTLSSNHASPLAAPTCT-RSRLRISATSTAAPSPSSAA
SEQ ID NO:04 MLLAKPHLSSSF-LPSTRVSSPAPGNHAKPIAASPAP-RRCLRLAVTSAAAPAAASSAE
SEQ ID NO:06 MMAG----AAKTLTNI.CPSFEPPTKPNQLTTSHPFSTRLRHRAISAVANAAPPLAAA
SEQ ID NO:08 MLLTKPH---PALTLPSASLPNPNLKAARVRPLASSAPHGRRGLRV---SASSSSLAPAQ
SEQ ID NO:12 MXXXXXXX
1
60

+      +
SEQ ID NO:11 --EAATRVKILSEALPYIQHFAGRTVVVVKYGGAAKDSNLKDKVIRDIVEMASVGIRPV
SEQ ID NO:02 AATASLSRVDVLSEALPFIQRFKGTVVVVKYGGAAKSPELQASVIRDLVLLSCVGLRPV
SEQ ID NO:04 AA-AALS RVDVLSEALPFIQRFKGTVVVVKYGGAAKSPELQASVIRDLVLLSCVGLHPV
SEQ ID NO:06 TATEGQYRVDVLSESLPFIQKFRGKTIVVVKYGGAAKSPELQASVIRDLVLLSCVGLRPV
SEQ ID NO:08 AASAALNRVDVLSEALPFIQRFKGTVVVVKYGGAAKSPELQASVIRDLVLLSCVGLRPV
SEQ ID NO:12 AXXXXXRVDVLSEALPFIQFXGKTXXVVKYGGAAKSPELQASVIXDLVLLSCVGLXPV
61
120

+      +      +
SEQ ID NO:11 VVHGGGPEINTWLDKVGIEPQFKDGLRVTDAATMDIVEMVLVGRVNKELVNLIQAGGKA
SEQ ID NO:02 LVHGGGPEINSWLLRVGVEPQFRDGLRVTDALTMVEVVEMVLVGKVNKNLVSLINIAGGTA
SEQ ID NO:04 LVHGGGPEINSWI.I.RVGVEPQFRNGLRVTDALNMEVVEMVI.VRKVNKEI.I.SI.IKIPGSSA
SEQ ID NO:06 LVHGGGPEINSWLGRNLNIPAVFRDGLRVTDAADTMEI.VSMVLVGKVNKT.LVSLINKAGATA
SEQ ID NO:08 LVHGGGPEINSWLQRVGVXPQFRNGLRVTXXXXXXXKQLLSLIRPAGTTA
SEQ ID NO:12 LVHGGGPEINSWLXXXXXXFRXGLRVTDAXXMEVXXMVLVXKVNKXLXSLIXXXGXXA
121
180

```

## FIGURE 1 (CONTINUED)

```

SEQ ID NO:11      *      +      ++      +      ++      +      ++      +      ++      +      ++      +      ++      +      ++      +
VGLCGKDGQLMTARTMTN-KDVGFEVGEVSSVDARVVETLVKSGYIPVISSVAADEFQGAH
SEQ ID NO:02      IGLCGKDARLITARPSPNAAALGFVGEVSRVDATVLHPHIIAAGHIPVIATVAADETGQAY
SEQ ID NO:04      VSLCWKEARLLNERPSPXKGLRFVGGVWRVDATVLHPHIIASGHIPVIATVGADETGQAY
SEQ ID NO:06      VGLSGMDGRLLTARPAPKAADLGYVGEVARVDPVLRSLIDTSHIPVVTSVAADESGQPY
SEQ ID NO:08      VGLCRKDGRIILTERPSPDAAALGFVGEVTRKNPSVLHPHIIASSHIPVIATVAADETGQAY
SEQ ID NO:12      XXLXXXXXRLXXXXRPPXXXXLXXVGVXVXXXXXXVXXXXXHIPVXXXXXVXADEXGQXY
181
*****      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
NINADTCAGELAAALGAEKLLLLTDRGILRDYKDPSTLIHKLDIQQARELIGSGIVAGG
SEQ ID NO:11
SEQ ID NO:02      NINADTAAGEIAAAVGAEKLLLLTDVSGILADRNPDGSLVKVVDIAGVRKMVADGKVAGG
SEQ ID NO:04      NINADTAAGEIAAAVGAEKLLLLTDVSGILADRNPDGSLVKEIDIAGVRQMVADGQVAGG
SEQ ID NO:06      NINADTVAGELAAASLGAEKLLLLTDVAGILEDRNDPDSLKKIDIKGVKKMMEDGKVGGG
SEQ ID NO:08      NINADTAAGEIAAAIGAEKLLLLITDVSILADRDGSLVKEIDIAGVRRMVAEGKVGGG
SEQ ID NO:12      NINADTXAGEXAAXXGAEKLLXLTDXVXGILXDRXDPXSLVKXXDIXGVRXMXXXGXVXGG
241
*****      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
MIPKVTCCVRSLAQGVRAAHILDGRLPHALLLEVFDTDLGIGSMIVASGYDL
SEQ ID NO:11
SEQ ID NO:02      MIPKVECCVHALAQGVHTASIIDGRVPHSLLEILTDEGTGMTG.-----
SEQ ID NO:04      MIPKVECCVRAIAQGVHTASIIDGRVPHSLLEILTDEGTGMTG.-----
SEQ ID NO:06      MIPKVNCCVRSLAQGVITASIIDGRVPHSLLEILTDEGAGTMTG.-----
SEQ ID NO:08      MIPKVGCCVRALAQGVHTASIIDGRVPHSLLEILTDEGTGMTG.-----
SEQ ID NO:12      MIPKVXCCVXXLAQGVXTASILDGRVPHSLLEILTDEGXGMTG
301

```

Rev. 06/95

Docket Number

BB1174

## DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**ORNITHINE BIOSYNTHESIS ENZYMES**

the specification of which is attached hereto unless the following box is checked:

☒ was filed on **14 JULY 1999** as U.S. Application No. \_\_\_\_\_ or PCT International Application No. **PCT/US99/15931** and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)
-----------------	---------	-------------	---------------------------

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.

U.S. Filing Date

60/093,209

17 JULY 1998

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)
-----------------	-------------	---

**POWER OF ATTORNEY:** I hereby appoint the following attorney(s) and/or agent(s) the power to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:Name: **WILLIAM R. MAJARIAN**Registration No.: **41,173**

Send correspondence and direct telephone calls to:

**WILLIAM R. MAJARIAN**
**E. I. du Pont de Nemours and Company**  
 Legal - Patents  
 Wilmington, DE 19898, U.S.A.
Tel. No.  
(302) 992-4926Fax No.  
(302) 892-7949

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

**INVENTOR(S)**

Full Name of Inventor	Last Name <b>CAHOON</b>	First Name <b>REBECCA</b>	Middle Name
	Signature (please sign full name): <i>Rebecca E. Cahoon</i>		Date: <i>August 23, 1999</i>
Residence & Citizenship	City <b>WILMINGTON</b>	State or Foreign Country <b>DELAWARE DE</b>	Country of Citizenship <b>U.S.A.</b>
Post Office Address	Post Office Address <b>2331 WEST 18TH STREET</b>	City <b>WILMINGTON</b>	State or Country <b>DELAWARE</b> Zip Code <b>19806</b>
Full Name of Inventor	Last Name <b>GUTTERIDGE</b>	First Name <b>STEVEN</b>	Middle Name
	Signature (please sign full name): <i>Steven Gutteridge</i>		Date: <i>August 24, 1999</i>
Residence & Citizenship	City <b>WILMINGTON</b>	State or Foreign Country <b>DELAWARE DE</b>	Country of Citizenship <b>USA</b>
Post Office Address	Post Office Address <b>4 AUSTIN ROAD</b>	City <b>WILMINGTON</b>	State or Country <b>DELAWARE</b> Zip Code <b>19810</b>
Full Name of Inventor	Last Name <b>LEE</b>	First Name <b>JIAN-MING</b>	Middle Name
	Signature (please sign full name): <i>Jian-Ming Lee</i>		Date: <i>Sept. 21, 1999</i>
Residence & Citizenship	City <b>WILMINGTON</b>	State or Foreign Country <b>DELAWARE DE</b>	Country of Citizenship <b>CN</b>
Post Office Address	Post Office Address <b>3120 NAAMANS ROAD, APARTMENT 203</b>	City <b>WILMINGTON</b>	State or Country <b>DELAWARE</b> Zip Code <b>19810</b>

☒ Additional Inventors are being named on separately numbered sheets attached hereto.

JAN 03 2001

**GENERAL POWER OF ATTORNEY**  
(Concerning Several International Patent Applications)

The undersigned, Vernon R. Rice, Vice President and Assistant General Counsel of E. I. DU PONT DE NEMOURS AND COMPANY, 1007 Market Street, Wilmington, Delaware 19898 USA ("DuPont"), hereby confirms that the power to sign for DuPont has been granted to various individuals (as set forth in the attached excerpt from DuPont's Patent Board Rules of Procedure (January 1988), Appendix Section III.A.4), including the Chairman, Vice-Chairman, and those individuals who are Assistant Secretaries of the Patent Board. Currently these Assistant Secretaries are:

Roger A. Bowman  
Linda J. Davis  
John E. Griffiths

Miriam D. McConnahey  
Dorothy W. Shafer  
Deborah A. Meginniss

In addition, the authority to act on behalf of DuPont before the competent International Authorities in connection with any and all international patent applications filed by it with the United States as Receiving Office and to make or receive payments on its behalf is hereby granted to:

Beardell, Lori Y.	<u>34,293</u>	Katz, Elliott A.	<u>26,396</u>
Belopolsky, Inna	<u>43,319</u>	Kelly, Patricia L.	<u>39,247</u>
Benjamin, Steven C.	<u>36,087</u>	King, Karen K.	<u>34,850</u>
Birch, Linda D.	<u>38,719</u>	Kuller, Mark D.	<u>31,925</u>
Bowen, Jr., Alanson G.	<u>24,027</u>	Krukziel, Charles E.	<u>27,344</u>
Christenbury, Lynne M.	<u>30,971</u>	Jarnholm, Arne R.	<u>30,396</u>
Cotreau, William J.	<u>36,490</u>	Langworthy, John A.	<u>32,255</u>
Deitch, Gerald E.	<u>30,457</u>	Lerman, Bart E.	<u>31,897</u>
Deshmukh, Sudhir	<u>33,677</u>	Levitt, Cary A.	<u>31,848</u>
Dobson, Kevin S.	<u>40,296</u>	Magee, Thomas H.	<u>27,355</u>
Duffy, Roseanne R.	<u>33,869</u>	Mayer, Nancy S.	<u>29,190</u>
Edwards, Mark A.	<u>39,542</u>	Medwick, George M.	<u>27,456</u>
Estrin, Barry	<u>26,452</u>	Morrissey, Bruce W.	<u>30,663</u>
Evans, Craig H.	<u>31,825</u>	Santopietro, Lois A.	<u>36,264</u>
Fair, Tamera L.	<u>35,867</u>	Schaeffer, Andrew L.	<u>33,605</u>
Feltham, S. Neil	<u>36,506</u>	Sebree, Chyrea J.	<u>45,348</u>
Floyd, Linda Axiemethy	<u>33,692</u>	Shafer, Robert J.	<u>24,437</u>
Frank, George A.	<u>27,636</u>	Shay, Lucas K.	<u>34,724</u>
Golian, Andrew G.	<u>25,293</u>	Shiple, James E.	<u>32,003</u>
Gorman, Thomas W.	<u>31,959</u>	Siegell, Barbara C.	<u>30,684</u>
Gould, David J.	<u>25,338</u>	Sinnott, Jessica M.	<u>34,015</u>
Griffiths, John E.	<u>32,647</u>	Steinberg, Thomas W.	<u>37,013</u>
Hamby, Jane O.	<u>32,872</u>	Stevenson, Robert B.	<u>26,039</u>
Hamby, William H.	<u>31,521</u>	Strickland, Frederick D.	<u>39,041</u>
Heiser, David E.	<u>31,366</u>	Tessari, Joseph A.	<u>32,177</u>
Hendrickson, John S.	<u>30,847</u>	Tulloch, Rebecca W.	<u>36,297</u>
Jones, Brian C.	<u>37,857</u>	Walker, P. Michael	<u>32,602</u>
Joung, J. Kenneth	<u>41,881</u>	Wang, Chen	<u>38,650</u>

The undersigned ratifies fully all actions already taken by the above-named individuals in accordance with the authority granted hereby.

E. I. DU PONT DE NEMOURS AND COMPANY

By: \_\_\_\_\_

Vernon R. Rice

Vice President and Assistant General Counsel

Date: \_\_\_\_\_

5/11/2000

10 Rec'd 09/744,100 01 JUN 2001

#4

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN THE APPLICATION OF:  
R. CAHOON ET AL.

CASE NO.: BB1174 PCT

APPLICATION NO.: 09/744,100

GROUP ART UNIT: UNKNOWN

FILED: JANUARY 16, 2001

EXAMINER: UNKNOWN

FOR: **ORNITHINE BIOSYNTHESIS ENZYMES**

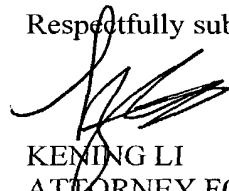
Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

**STATEMENT UNDER 37 CFR 1.821(g) and 1.825(b)**

The submission of the substitute computer readable Sequence Listing filed concurrently herewith does not include new matter.

Respectfully submitted,



KENING LI  
ATTORNEY FOR APPLICANTS  
REGISTRATION NO. 44,872  
TELEPHONE: 302-992-3749  
FACSIMILE: 302-892-1026

Dated: 05/31/2001

WO 00/04168

SEQUENCE LISTING

<110> Cahoon, Rebecca E.  
Gutteridge, Steven  
Lee, Jian Ming  
Rafalski, Antoni

<120> Ornithine Biosynthesis Enzymes

<130> BB-1174

<140>

<141>

<150> 60/093,209

<151> July 17, 1998

<160> 12

<170> Microsoft Office 97

<210> 1

<211> 1201

<212> DNA

<213> Zea mays

<400> 1

```

tcgagctcga gctcagagccc cagtcaccgc agccatgctc ctcacgaaac cctacctctc 60
caactcgctc cttccagtc ccatccccgc gccgtcgggc cctactctca gctccaaacca 120
tgcaagcccc cttgccgccc ctacttgccg tcgcagccgc ctccgcatct ccgccacatc 180
cacggctgcg ccgtctcctt cgtcggctgc cgtgccacc gcgtcgtga gtcgagtga 240
cgtgctctcg gaggcgctcc cttttattca gcgattcaaa ggcaagacgg tgggtgtcaa 300
gtacggcggt gccggcgatga agtccccgga gctgcaggcg tccgtgatcc gcgatctcgt 360
gctgctctcc tgcgtcgggc tccgccccgt gcttggtcac ggcgggcggtc cggagattaa 420
ttcttggtcg ctgcgcgtcg gcgtcgagcc gcagttccgc gacggcctcc gcgtcacgga 480
cgcgctcacc atggaggtcg tcgagatggt gctagtcggg aaggtcaaca aaaaccttgt 540
ttccctcatc aacatcgcgg gaggcaccgc cattggtctg tgcggcaagg acgcgcgcct 600
tatcaccgct cgcctctctc caaatgcagc ggcgctggga ttctgcggcg aggtttcgcg 660
cgtggacgcc accgtctctc atcccacat cgcgcggggc catatcccg ttatcgccac 720
cgttgccgcc gacgagactg ggcaagccta taacatcaat gctgatacgg cggctggcga 780
gattgccgct gccgtgggcg ccgagaagct gctggtgctc acagatgtgt cggcatttt 840
ggcggaccgt aatgacctg ggagcctggt gaaggtggtc gacattgctg cgggtcgga 900
gatggtggt gacgggaagg tagctggtgg gatgataccc aaggtggagt gttgtgttca 960
cgcccttgca caaggtgtac acaccgcaag tatcattgat gggcgtgttc cacactctct 1020
tctgcttgag attctcacag acgagggcac aggcaccatg atcactggct gagctgcttc 1080
atgccttcat ggtattttcc tgtgcctctt ttctcatatt gttgtgtttt atggctatgt 1140
agactaaact caagattgca ataagactac ctaagtttgg ttgaaaaaaa aaaaaaaaaa 1200
a 1201

```

<210> 2

<211> 345

<212> PRT

<213> Zea mays

<400> 2

```

Met Leu Leu Thr Lys Pro Tyr Leu Ser Asn Ser Leu Leu Pro Val Pro
1 5 10 15

```

```

Ser Pro Pro Pro Ser Gly Pro Thr Leu Ser Ser Asn His Ala Ser Pro
20 25 30

```



WO 00/04168

PCT/US99/15931

Leu Ala Ala Pro Thr Cys Arg Arg Ser Arg Leu Arg Ile Ser Ala Thr  
35 40 45

Ser Thr Ala Ala Pro Ser Pro Ser Ser Ala Ala Ala Ala Thr Ala Ser  
50 55 60

Leu Ser Arg Val Asp Val Leu Ser Glu Ala Leu Pro Phe Ile Gln Arg  
65 70 75 80

Phe Lys Gly Lys Thr Val Val Val Lys Tyr Gly Gly Ala Ala Met Lys  
85 90 95

Ser Pro Glu Leu Gln Ala Ser Val Ile Arg Asp Leu Val Leu Leu Ser  
100 105 110

Cys Val Gly Leu Arg Pro Val Leu Val His Gly Gly Gly Pro Glu Ile  
115 120 125

Asn Ser Trp Leu Leu Arg Val Gly Val Glu Pro Gln Phe Arg Asp Gly  
130 135 140

Leu Arg Val Thr Asp Ala Leu Thr Met Glu Val Val Glu Met Val Leu  
145 150 155 160

Val Gly Lys Val Asn Lys Asn Leu Val Ser Leu Ile Asn Ile Ala Gly  
165 170 175

Gly Thr Ala Ile Gly Leu Cys Gly Lys Asp Ala Arg Leu Ile Thr Ala  
180 185 190

Arg Pro Ser Pro Asn Ala Ala Ala Leu Gly Phe Val Gly Glu Val Ser  
195 200 205

Arg Val Asp Ala Thr Val Leu His Pro Ile Ile Ala Ala Gly His Ile  
210 215 220

Pro Val Ile Ala Thr Val Ala Ala Asp Glu Thr Gly Gln Ala Tyr Asn  
225 230 235 240

Ile Asn Ala Asp Thr Ala Ala Gly Glu Ile Ala Ala Ala Val Gly Ala  
245 250 255

Glu Lys Leu Leu Leu Leu Thr Asp Val Ser Gly Ile Leu Ala Asp Arg  
260 265 270

Asn Asp Pro Gly Ser Leu Val Lys Val Val Asp Ile Ala Gly Val Arg  
275 280 285

Lys Met Val Ala Asp Gly Lys Val Ala Gly Gly Met Ile Pro Lys Val  
290 295 300

Glu Cys Cys Val His Ala Leu Ala Gln Gly Val His Thr Ala Ser Ile  
305 310 315 320

Ile Asp Gly Arg Val Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp  
325 330 335

Glu Gly Thr Gly Thr Met Ile Thr Gly  
340 345



WO 00/04168

PCT/US99/15931

Gly Leu His Pro Val Leu Val His Gly Gly Gly Pro Glu Ile Asn Ser  
115 120 125

Trp Leu Leu Arg Val Gly Val Glu Pro Gln Phe Arg Asn Gly Leu Arg  
130 135 140

Val Thr Asp Ala Leu Asn Met Glu Val Val Glu Met Val Leu Val Arg  
145 150 155 160

Lys Val Asn Lys Glu Leu Leu Ser Leu Ile Lys Leu Pro Gly Gly Ser  
165 170 175

Ala Val Ser Leu Cys Trp Lys Glu Ala Arg Leu Leu Asn Glu Arg Pro  
180 185 190

Ser Pro Xaa Glu Lys Gly Leu Arg Phe Val Gly Gly Val Trp Arg Val  
195 200 205

Asp Ala Thr Val Leu His Pro Ile Ile Ala Ser Gly His Ile Pro Val  
210 215 220

Ile Ala Thr Val Gly Ala Asp Glu Thr Gly Gln Ala Tyr Asn Ile Asn  
225 230 235 240

Ala Asp Thr Ala Ala Gly Glu Ile Ala Ala Ala Val Gly Ala Glu Lys  
245 250 255

Leu Leu Leu Leu Thr Asp Val Ser Gly Ile Leu Ala Asp Arg Asn Asp  
260 265 270

Pro Gly Ser Leu Val Lys Glu Ile Asp Ile Ala Gly Val Arg Gln Met  
275 280 285

Val Ala Asp Gly Gln Val Ala Gly Gly Met Ile Pro Lys Val Glu Cys  
290 295 300

Cys Val Arg Ala Leu Ala Gln Gly Val His Thr Ala Ser Ile Ile Asp  
305 310 315 320

Gly Arg Val Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp Glu Gly  
325 330 335

Thr Gly Thr Met Ile Thr Gly  
340

<210> 5  
<211> 1204  
<212> DNA  
<213> Glycine max

<400> 5  
gcacgagatg atggcaggtg cagccaaaac cctaaccaat ctttgcccct ctttcccatt 60  
cccaaccaaa ccccaaaacc aactcaccac tagccacgc tcccttcca ctcgcctccg 120  
ccacgcgcgc atttcgcggg tggcgaacgc ggcgcaacct ccactgcgc cgcgcaactgc 180  
caccgagggt cagtaaccgag tcgatgtgct ctccgagtc ctcaccttca tccagaaatt 240  
ccgcggcaaaa accatcgtcg tcaagtacgg cggcgccgc atgaagtccc cggagctcca 300  
ggcctccgtg atcaacgacc ttgtcctcct ctcccgctc ggccctccgc cgtcctggt 360  
ccacggcggc ggcccgaga tcaactcctg gtcgggcgc ctcaacatcc cgcgcgtctt 420  
ccgcgaacggc ctccggtca ccgacgccga caccatggag atcgtctcca tggctcctgt 480  
cggaaaagtc aacaaaacc tagtttctct aattaacaag gccggcgcca ccgcgcgtcg 540

WO 00/04168

PCT/US99/15931

```

cctctctggc atggacggcc gcctccacac cgcgcgcgcg gctcccaagg ccgcccagct 600
cggctacgtc ggcgaggtcg cagcgcgcga tcccgcgcgc ctcgcgtccc taatcgacac 660
cagccacatc ccgcgcgtca cctccgcgcg cgcgcgatgaa tccggacagc cctacaacat 720
caacgcgcgac accgcgcgcg gagaattggc agcgcgcgcg ggcgcggaga agctgattct 780
gctgaccgat gtggcgggaa ttctggaaga tcggaacgac cctgacagct cggatgaaga 840
gattgacata aaaggagtga agaaaatgat ggaagatgga aaagtgggtg ggggaatgat 900
acctaagggtt aattggtgcg ttaggtcctt ggcgcgaagg gttattacag cgagtattat 960
tgatggtagg gttccgcatt ctttgttgct tgagattttg actgatgaag gtcgtggaac 1020
tatgataact ggataagttt atttatttat ggtggttgga tttttctttt caatcaagc 1080
cttgagttga ggttgacatt cagcacttgt tttgttagag attggtgatt gtttttaagt 1140
gcgtgtaatg tgagagatgg ttgaattgaa ttgaatgttt cagaaaaaaaa aaaaaaaaaa 1200
aaaa 1204

```

<210> 6  
 <211> 342  
 <212> PRT  
 <213> Glycine max

<400> 6  
 Met Met Ala Gly Ala Ala Lys Thr Leu Thr Asn Leu Cys Pro Ser Phe  
 1 5 10 15  
 Pro Phe Pro Thr Lys Pro Gln Asn Gln Leu Thr Thr Ser His Ala Phe  
 20 25 30  
 Pro Ser Thr Arg Leu Arg His Arg Ala Ile Ser Ala Val Ala Asn Ala  
 35 40 45  
 Ala Gln Pro Pro Leu Ala Ala Ala Thr Ala Thr Glu Gly Gln Tyr Arg  
 50 55 60  
 Val Asp Val Leu Ser Glu Ser Leu Pro Phe Ile Gln Lys Phe Arg Gly  
 65 70 75 80  
 Lys Thr Ile Val Val Lys Tyr Gly Gly Ala Ala Met Lys Ser Pro Glu  
 85 90 95  
 Leu Gln Ala Ser Val Ile Asn Asp Leu Val Leu Leu Ser Cys Val Gly  
 100 105 110  
 Leu Arg Pro Val Leu Val His Gly Gly Gly Pro Glu Ile Asn Ser Trp  
 115 120 125  
 Leu Gly Arg Leu Asn Ile Pro Ala Val Phe Arg Asp Gly Leu Arg Val  
 130 135 140  
 Thr Asp Ala Asp Thr Met Glu Ile Val Ser Met Val Leu Val Gly Lys  
 145 150 155 160  
 Val Asn Lys Thr Leu Val Ser Leu Ile Asn Lys Ala Gly Ala Thr Ala  
 165 170 175  
 Val Gly Leu Ser Gly Met Asp Gly Arg Leu Leu Thr Ala Arg Pro Ala  
 180 185 190  
 Pro Lys Ala Ala Asp Leu Gly Tyr Val Gly Glu Val Ala Arg Val Asp  
 195 200 205  
 Pro Ala Val Leu Arg Ser Leu Ile Asp Thr Ser His Ile Pro Val Val  
 210 215 220

WO 00/04168

PCT/US99/15931

Thr Ser Val Ala Ala Asp Glu Ser Gly Gln Pro Tyr Asn Ile Asn Ala  
 225 230 235 240  
 Asp Thr Val Ala Gly Glu Leu Ala Ala Ser Leu Gly Ala Glu Lys Leu  
 245 250 255  
 Ile Leu Leu Thr Asp Val Ala Gly Ile Leu Glu Asp Arg Asn Asp Pro  
 260 265 270  
 Asp Ser Leu Val Lys Lys Ile Asp Ile Lys Gly Val Lys Lys Met Met  
 275 280 285  
 Glu Asp Gly Lys Val Gly Gly Gly Met Ile Pro Lys Val Asn Cys Cys  
 290 295 300  
 Val Arg Ser Leu Ala Gln Gly Val Ile Thr Ala Ser Ile Ile Asp Gly  
 305 310 315 320  
 Arg Val Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp Glu Gly Ala  
 325 330 335  
 Gly Thr Met Ile Thr Gly  
 340

<210> 7  
 <211> 1246  
 <212> DNA  
 <213> Triticum aestivum  
 <220>  
 <221> unsure  
 <222> (492)..(542)

<400> 7  
 gcacgaggtc taggcaagca acccggcggc gcgaacacca agtccccccg gcgggccgcag 60  
 caatgtcctt aaccaagccc caccgcgcc tcacctccc ctccgcatcc ctcccaaate 120  
 ctaacctaaa ggccgcccgc gtcaggcccc tgcctctctc cgcgcccat ggacgcgcgc 180  
 ggctccgcgt ctgcgcctcc tctctctccc tggcgccagc gcaggccgcg tccgcggcgc 240  
 tgaaccgcgt ggacgtcctg tcggaggcgc tcccttctat ccagcggttc aagggaaga 300  
 cgggtggtgg caagtacggc ggcgcgcca tgaagtcgcc ggagctgcag gcgtcggtga 360  
 tccgcgacct ggtctctctc tctgcgtcgc gcctgcgcgc cgtgctcgtg cacggcggcg 420  
 gcccgagat caactcctgg ctgcagcgcg tcggggtcta gccgcagttc cgcaacgggc 480  
 tccgcgtcac gnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 540  
 nnaagcagct cttatcccta atcaggcctg cggggaccac agcagttggc ctctgcagaa 600  
 aggacgggcg catcctaacg gacgcgccct cccagacgc cgacgcctc gggttcgtcg 660  
 gcgaggtcac gagaaaaaac ccctctgtgc tccaccgat catgcctcc agccacatcc 720  
 cggctatcgc caccgtggct gccgacgaga ccgggcaagc ctataacatc aacgctgaca 780  
 ctgcggcggg ggagatcgcg gctgccattg gcgcccagaa gctgttgctg atcactgacg 840  
 tgtccggcat actcgcggac cgggatgacc ccgggagcct ggtgaaggag attgacatcg 900  
 ccggcgtagc gcggatggtg gccgagggca aggtgggtgg gggcatgata cccaagggtg 960  
 ggtgctgcgt gcgcgcgctg gcgcaggcgc tgcacacggc cagcatcatt jacggccgcg 1020  
 tcccgcaactc tctctgctc gaaatcctca ccgacgaggg caccggcacc atgatcaccg 1080  
 gctgaaactt gttgtttgt tttgttttt ttctttctt ttttggttca cattctttgg 1140  
 gttggatggt tttgatccc tcatttgtgt taaatgtgt ttcgattcga atcctgaaca 1200  
 aggagtgggt aaagattgca gctttaagca aaaaaaaaa aaaaaa 1246

<210> 8  
 <211> 340

WO 00/04168

PCT/US99/15931

<212> PRT  
 <213> Triticum aestivum

<220>  
 <221> UNSURE  
 <222> (133)

<220>  
 <221> UNSURE  
 <222> (144)..(160)

<400> 8  
 Met Leu Leu Thr Lys Pro His Pro Ala Leu Thr Leu Pro Ser Ala Ser  
 1 5 10 15  
 Leu Pro Asn Pro Asn Leu Lys Ala Ala Arg Val Arg Pro Leu Ala Ser  
 20 25 30  
 Ser Ala Pro His Gly Arg Arg Gly Leu Arg Val Ser Ala Ser Ser Ser  
 35 40 45  
 Ser Leu Ala Pro Ala Gln Ala Ala Ser Ala Ala Leu Asn Arg Val Asp  
 50 55 60  
 Val Leu Ser Glu Ala Leu Pro Phe Ile Gln Arg Phe Lys Gly Lys Thr  
 65 70 75 80  
 Val Val Val Lys Tyr Gly Gly Ala Ala Met Lys Ser Pro Glu Leu Gln  
 85 90 95  
 Ala Ser Val Ile Arg Asp Leu Val Leu Leu Ser Cys Val Gly Leu Arg  
 100 105 110  
 Pro Val Leu Val His Gly Gly Gly Pro Glu Ile Asn Ser Trp Leu Gln  
 115 120 125  
 Arg Val Gly Val Xaa Pro Gln Phe Arg Asn Gly Leu Arg Val Thr Xaa  
 130 135 140  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 145 150 155 160  
 Lys Gln Leu Leu Ser Leu Ile Arg Pro Ala Gly Thr Thr Ala Val Gly  
 165 170 175  
 Leu Cys Arg Lys Asp Gly Arg Ile Leu Thr Glu Arg Pro Ser Pro Asp  
 180 185 190  
 Ala Ala Ala Leu Gly Phe Val Gly Glu Val Thr Arg Lys Asn Pro Ser  
 195 200 205  
 Val Leu His Pro Ile Ile Ala Ser Ser His Ile Pro Val Ile Ala Thr  
 210 215 220  
 Val Ala Ala Asp Glu Thr Gly Gln Ala Tyr Asn Ile Asn Ala Asp Thr  
 225 230 235 240  
 Ala Ala Gly Glu Ile Ala Ala Ala Ile Gly Ala Glu Lys Leu Leu Leu  
 245 250 255

WO 00/04168

PCT/US99/15931

Ile Thr Asp Val Ser Gly Ile Leu Ala Asp Arg Asp Asp Pro Gly Ser  
 260 265 270  
 Leu Val Lys Glu Ile Asp Ile Ala Gly Val Arg Arg Met Val Ala Glu  
 275 280 285  
 Gly Lys Val Gly Gly Gly Met Ile Pro Lys Val Gly Cys Cys Val Arg  
 290 295 300  
 Ala Leu Ala Gln Gly Val His Thr Ala Ser Ile Ile Asp Gly Arg Val  
 305 310 315 320  
 Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp Glu Gly Thr Gly Thr  
 325 330 335  
 Met Ile Thr Gly  
 340

<210> 9  
 <211> 439  
 <212> DNA  
 <213> Triticum aestivum

<400> 9  
 gcacgaggtg agattgccgc tgcggtgggc gccgagaaat tgctactgct cacagatgtg 60  
 tctgggatac tggcggaccg taatgaccct ggcagcctgg tgaaggagat tgacatcgct 120  
 ggggtgcggc agatgggtatc cggtgggcag gttgctgggt gaatgatccc aaagggtggag 180  
 tgctgcgtga gagccctcgc ccagggtgtg cacactgcaa gcatcatcga tgggcgtgtc 240  
 ccgcactcgc tgttgctcga gattctcaca gatgagggca ctggcacaat gatcaccggc 300  
 taagggtgtaa aatgcctcct tgggtacttcc ttatgccttt ctgttcatac tgccaatctg 360  
 ccatgtaatt tatgccaatg tagcctcacc tcatgattgc aataagagta ccttcctgac 420  
 aaaaaaaaaa aaaaaaaaaa 439

<210> 10  
 <211> 100  
 <212> PRT  
 <213> Triticum aestivum

<400> 10  
 Ala Arg Gly Glu Ile Ala Ala Ala Val Gly Ala Glu Lys Leu Leu Leu  
 1 5 10 15  
 Leu Thr Asp Val Ser Gly Ile Leu Ala Asp Arg Asn Asp Pro Gly Ser  
 20 25 30  
 Leu Val Lys Glu Ile Asp Ile Ala Gly Val Arg Gln Met Val Ser Gly  
 35 40 45  
 Gly Gln Val Ala Gly Gly Met Ile Pro Lys Val Glu Cys Cys Val Arg  
 50 55 60  
 Ala Leu Ala Gln Gly Val His Thr Ala Ser Ile Ile Asp Gly Arg Val  
 65 70 75 80  
 Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp Glu Gly Thr Gly Thr  
 85 90 95  
 Met Ile Thr Gly  
 100





Met Ile Val Ala Ser Gly Tyr Asp Leu  
290 295

```
<210> 12
<211> 345
<212> PRT
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence:CONSensus

<400>															
Met	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
1				5					10					15	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			20					25					30		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
		35					40					45			
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Ala	Xaa	Xaa	Xaa
	50					55					60				
Xaa	Xaa	Arg	Val	Asp	Val	Leu	Ser	Glu	Xaa	Leu	Pro	Phe	Ile	Gln	Xaa
65					70					75					80
Phe	Xaa	Gly	Lys	Thr	Xaa	Val	Val	Lys	Tyr	Gly	Gly	Ala	Ala	Met	Lys
				85					90					95	
Ser	Pro	Glu	Leu	Gln	Ala	Ser	Val	Ile	Xaa	Asp	Leu	Val	Leu	Leu	Ser
			100					105					110		
Cys	Val	Gly	Leu	Xaa	Pro	Val	Leu	Val	His	Gly	Gly	Gly	Pro	Glu	Ile
		115					120					125			
Asn	Ser	Trp	Leu	Xaa	Arg	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Phe	Arg	Xaa	Gly
	130					135						140			
Leu	Arg	Val	Thr	Asp	Ala	Xaa	Xaa	Met	Glu	Xaa	Val	Xaa	Met	Val	Leu
145					150					155					160
Val	Xaa	Lys	Val	Asn	Lys	Xaa	Leu	Xaa	Ser	Leu	Ile	Xaa	Xaa	Xaa	Gly
				165					170					175	
Xaa	Xaa	Ala	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	Arg	Leu	Xaa	Xaa	Xaa
			180					185					190		
Arg	Pro	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Val	Gly	Xaa	Val	Xaa
		195					200					205			
Arg	Xaa	Xaa	Xaa	Xaa	Val	Leu	Xaa	Xaa	Xaa	Ile	Xaa	Xaa	Xaa	His	Ile
	210					215					220				
Pro	Val	Xaa	Xaa	Xaa	Val	Xaa	Ala	Asp	Glu	Xaa	Gly	Gln	Xaa	Tyr	Asn
225					230					235					240
Ile	Asn	Ala	Asp	Thr	Xaa	Ala	Gly	Glu	Xaa	Ala	Ala	Xaa	Xaa	Gly	Ala
				245					250					255	

WO 00/04168

PCT/US99/15931

Glu Lys Leu Xaa Leu Xaa Thr Asp Val Xaa Gly Ile Leu Xaa Asp Arg	
260	265 270
Xaa Asp Pro Xaa Ser Leu Val Lys Xaa Xaa Asp Ile Xaa Gly Val Arg	
275	280 285
Xaa Met Xaa Xaa Xaa Gly Xaa Val Xaa Gly Gly Met Ile Pro Lys Val	
290	295 300
Xaa Cys Cys Val Xaa Xaa Leu Ala Gln Gly Val Xaa Thr Ala Ser Ile	
305	310 315 320
Leu Asp Gly Arg Val Pro His Ser Leu Leu Leu Glu Ile Leu Thr Asp	
325	330 335
Glu Gly Xaa Gly Thr Met Ile Thr Gly	
340	345